

REVIEW

Pseudoactinomycotic radiate granules of the gynaecological tract: review of a diagnostic pitfall

B Pritt, S L Mount, K Cooper, H Blaszyk

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The filamentous bacterium actinomyces can cause serious gynaecological tract infections, including pelvic inflammatory disease (PID) and tubo-ovarian abscess. Thus, definitive diagnosis of actinomycotic granules (AMGs) in gynaecological specimens is clinically important. Non-infectious pseudoactinomycotic radiate granules (PAMRAGs) can mimic the microscopic appearance of AMGs. PAMRAGs may be more common than actinomycotic infections in specimens from patients using intrauterine devices and may be seen in patients with PID. Although the composition and aetiology of PAMRAGs is unclear and variable, a panel of histochemical stains can aid in diagnosis. On haematoxylin and eosin (H&E) stained sections, AMGs show as distinct granules with basophilic peripheral radiating filaments and a dense central eosinophilic core, whereas H&E stained sections of PAMRAGs feature refractile granules with irregular club-like peripheral projections and no central dense core. The filaments of AMGs are Gram positive on Brown and Brenn (B&B) stain and are highlighted with Gomori methenamine silver stain (GMS). They stain negatively with a modified acid fast bacillus (AFB) stain, aiding in the distinction of actinomyces from nocardia. PAMRAGs show negative or non-specific staining with B&B, GMS, and AFB stains. Therefore, knowledge of these staining properties and the distinguishing characteristics of PAMRAGs and AMGs enables recognition of this important diagnostic pitfall.

examination, with culture recommended for confirmation.

"Because complications of actinomycotic infection include tubo-ovarian abscess and pelvic inflammatory disease, it is important that this entity is correctly diagnosed in pathology specimens from the gynaecological tract"

Other substances found in gynaecological specimens can mimic the microscopic appearance of actinomyces and present a diagnostic pitfall for pathologists. These pseudoactinomycotic radiate granules (PAMRAGs), also known as pseudosulfur granules and radiate pseudocolonies, are non-pathogenic and must be distinguished from true AMGs.

INCIDENCE AND EPIDEMIOLOGY

PAMRAGs were classically identified in uterine curettages from patients using an IUD.^{3–7} In an early study, O'Brien *et al* examined 235 endometrial specimens obtained at the time of IUD removal.⁴ Of these, PAMRAGs were identified in 17 and AMGs in only one; this suggests that PAMRAGs are more common than true actinomycotic infections in specimens from that anatomical site. Similarly, Striepecke and Bollmann⁷ studied 123 endometrial curettages from women with IUDs and found PAMRAGs in 14 (11.4%), but they did not report the prevalence of AMGs in their series. Most recently, Padberg *et al* studied 100 consecutive endometrial curettages obtained from 100 women with an IUD at the time of device removal.⁶ Four samples showed actinomyces, 11 revealed PAMRAGs, and two were positive for both actinomyces and PAMRAGs. This shows that the diagnosis of PAMRAGs does not preclude the presence of true AMGs, and the pathologist must carefully examine the entire specimen for microorganisms.

Adding further diagnostic dilemma is the fact that PAMRAGs may be seen in the setting of PID. Horn and Bilek³ undertook a seven year retrospective review of consecutive endometrial curettages obtained from patients with a clinical diagnosis of PID at a large tertiary care facility in Germany. Among 55 patients with tubo-ovarian abscess, five harboured actinomyces and three

Filamentous bacteria from the genus actinomyces are often found in the female gynaecological tract, and are strongly associated with the use of intrauterine devices (IUDs).¹ These Gram positive, non-spore forming, anaerobic rods may thrive in locations such as the oral cavity and genitourinary tract.² The bacterial colonies form distinct actinomycotic granules (AMGs), referred to as sulfur granules because of their tan to yellow colour on gross examination. Microscopically, these granules are composed of dense aggregates of the filamentous bacteria. Because complications of actinomycotic infection include tubo-ovarian abscess and pelvic inflammatory disease (PID),¹ it is important that this entity is correctly diagnosed in pathology specimens from the gynaecological tract. The diagnosis may be suggested by histological

See end of article for authors' affiliations

Correspondence to: Dr H Blaszyk, Department of Pathology, University of Vermont College of Medicine, 89 Beaumont Avenue, Burlington, VT 05405, USA; hagen.blaszyk@vtmednet.org

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Abbreviations: AMG, actinomycotic granule; IUD, intrauterine device; PAMRAG, pseudoactinomycotic radiate granule; PID, pelvic inflammatory disease; TEM, transmission electron microscopy

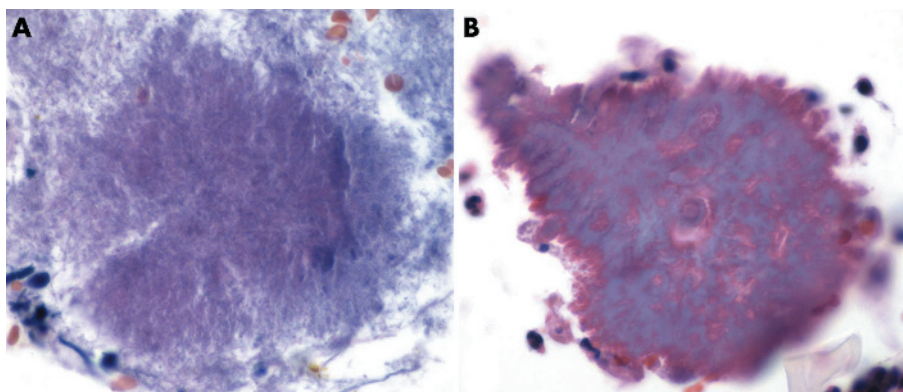


Figure 1 Although haematoxylin and eosin (H&E) staining shows some similarity between (A) actinomycotic granules (AMGs) and (B) pseudoactinomycotic radiate granules (PAMRAGs), some distinguishing features can be seen. AMGs comprise irregular to spherical, non-refractile granules with peripheral thin filaments and an eosinophilic granular dense centre. In contrast, PAMRAGs comprise irregular spherical granules and strips of crystalline, refractile material without a central dense core. H&E staining; original magnification, $\times 60$.

specimens contained PAMRAGs. All patients had an IUD with an average duration of 9.8 years.

Despite the association with IUD, morphologically identical granules have been identified in patients who have never used an IUD. In a descriptive study, Bhagavan *et al* identified and characterised six cases of PAMRAGs.⁸ Three of these cases were identified from endometrial curettings in patients using an IUD. The remaining three cases were seen in endocervical glands and nabothian cysts in patients who underwent hysterectomy for leiomyomata. These patients had never used an IUD or vaginal pessary.

HISTOLOGY

Actinomycotic granules

On routine haematoxylin and eosin staining, actinomycotic colonies are seen as distinct non-refractile granules, with thin basophilic radiating filaments at the periphery and a dense finely granular appearing central core (fig 1). The core may appear slightly more eosinophilic than the rest of the granule. The filaments are Gram positive on Brown and Brenn tissue Gram stain, and are highlighted with Gomori methenamine silver stain (figs 2, 3). AMGs do not stain with a modified acid fast bacillus preparation, aiding in their distinction from the filamentous bacteria *Nocardia*. Although the presence of sulfur granules is considered pathognomonic for actinomyces, there are other species of Gram positive filamentous bacteria present in the oral cavity and gynaecological tract; thus, culture is recommended for definitive diagnosis.

Pseudoactinomycotic radiate granules

In contrast to the thin filaments of AMGs, haematoxylin and eosin stained sections of PAMRAGs demonstrate thick irregular club-like peripheral projections without a central dense core (fig 1). Some granules are spherical, whereas others appear as strips, with club-like projections along one

aspect. An associated inflammatory response may be present in the form of surrounding neutrophils. Although PAMRAGs have a refractile appearance, they are non-birefringent with polarised light. The Brown and Brenn stain exhibits diffuse intense non-specific staining in PAMRAGs (fig 2), whereas silver stains for fungal organisms are negative (fig 3). Stains for acid fast bacillus are also negative or show a non-specific pattern. Table 1 summarises the expected results of this histochemical panel for both AMGs and PAMRAGs.

CYTOLOGY

Objects resembling PAMRAGs have been reported in cervicovaginal smears, where they have been termed “crystalline bodies” or “haematoidin cockleburs”.⁹ Zaharopoulos *et al* reviewed 35 000 smears, and found 111 crystalline bodies with a radiate structure, most of which were found in smears from pregnant women.¹⁰ The morphological, histochemical, and immunohistochemical phenotype of these structures closely resembles that seen in PAMRAGs, suggesting that “crystalline bodies” and PAMRAGs are identical structures. However, PAMRAGs were morphologically different from haematoidin cockleburs, which consist of radially arranged, fine, thin, weakly birefringent needles with a bilious golden colour that is not altered by routine staining.¹⁰ Therefore, haematoidin cockleburs are probably not related to PAMRAGs.

COMPOSITION AND AETIOLOGY

Some investigators have likened PAMRAGs to the Splendore–Hoeppli phenomenon.^{6–8} In 1908, Splendore identified and described eosinophilic material surrounding bacteria in a biopsy and erroneously believed that he had discovered a new species of sporotrichum.¹¹ Several years later, Hoeppli described similar eosinophilic material surrounding schistosomes in an

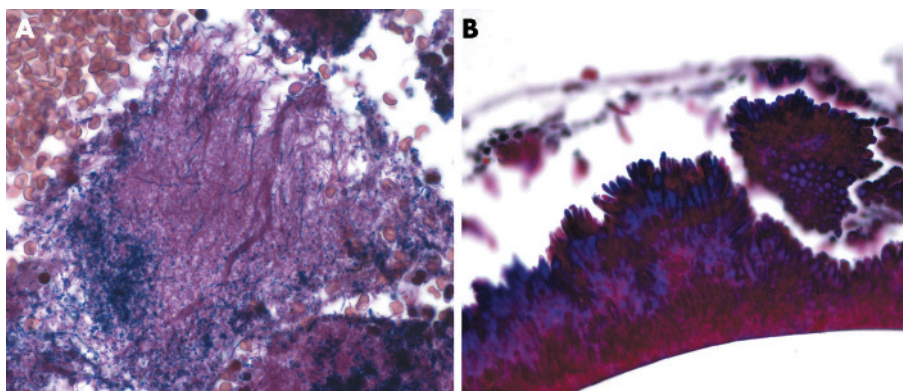


Figure 2 (A) Tissue Gram stain highlights the Gram positive filamentous bacteria in actinomycotic granules, and (B) shows strong non-specific staining in pseudoactinomycotic radiate granules. Brown and Brenn staining; original magnification, $\times 60$.

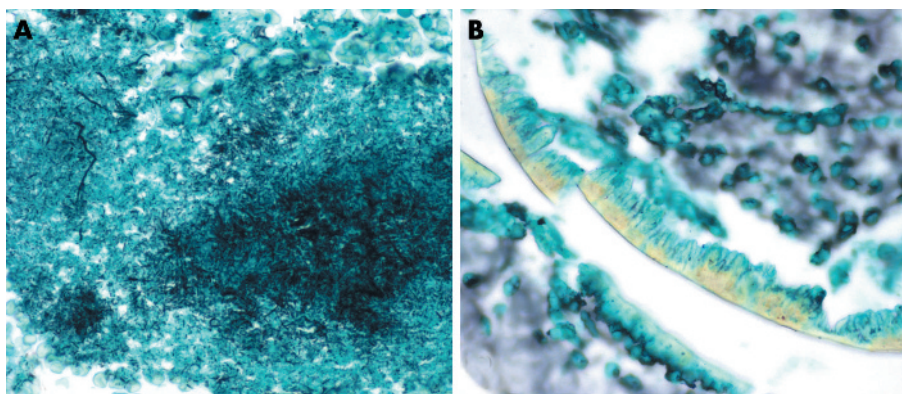


Figure 3 (A) Silver staining highlights the filamentous bacteria forming an actinomycotic granule and (B) is completely negative in pseudoactinomycotic radiate granules. Gomori methenamine silver staining; original magnification, $\times 60$.

experimental rabbit model and felt they were secretions of miracidia.¹² The Splendore–Hoepli phenomenon appears as radiating stellate or concentric eosinophilic material surrounding a variety of organic and inorganic substances, and is now thought to represent a host mediated immune response. Most often, it is associated with the presence of microorganisms, including actinomyces; thus, the use of this term to describe PAMRAGs may be confusing.

“Although it is now clear that pseudoactinomycotic radiate granules do not contain microorganisms, the exact composition and nature of these granules is debateable”

The non-infectious nature of PAMRAGs is supported by microbiology culture, histochemical studies, and ultrastructural analysis. In their original paper, O'Brien *et al* subjected 17 cases of PAMRAGs to Gram stain, Ziehl–Neelsen, periodic acid Schiff, and Grocott's modification of the Gomori's methenamine silver stain, but failed to identify bacterial or fungal elements.⁴ This histochemical analysis for microorganisms has been confirmed by other investigators.^{3–6–8} O'Brien *et al* also undertook a prospective study of 50 IUDs cultured immediately after removal.⁴ As would be expected, the cultures yielded a variety of organisms, including normal vaginal flora in 17 cases. Nineteen cultures grew organisms not considered to be part of the usual vaginal flora, including aerobic and anaerobic bacteria and fungi (*Candida albicans* and *Penicillium* spp.). Actinomyces species were not identified in the cultures and there was no growth in 14 cases. Review of the corresponding endometrial curettings obtained at the time of IUD removal revealed three cases of PAMRAGs. In these three cases, two IUD cultures grew vaginal flora and one grew penicillium species. Based on these results, the authors concluded that PAMRAGs are not associated with a specific infectious process. Further study by transmission

electron microscopy (TEM)^{5–7} confirmed the absence of microbial elements.

Although it is now clear that PAMRAGs do not contain microorganisms, the exact composition and nature of these granules is debateable. O'Brien *et al* originally postulated that PAMRAGs represent dissociated IUD fragments.⁴ This hypothesis was revised after the discovery of PAMRAGs in patients without IUDs,^{5–8} and analysis by TEM that did not demonstrate IUD elements.⁵ Through histochemical analysis, Bhagavan *et al* identified lipid, calcium, and neutral glycoproteins in PAMRAGs.⁸ Further immunohistochemical analysis did not identify fibrin, immunoglobulin, or complement.⁸ Based on this analysis, these authors concluded that PAMRAGs were not formed by an immune mediated process involving antigen–antibody complexes, and suggested that they represent a non-specific host leucocyte response to foreign bodies, parasites, or bacteria. Similarly, Striepecke and Bollmann⁷ suggested that the induction of increased lysosomal activity by exogenous factor(s) may trigger the formation of a nidus and result in the formation of PAMRAGs. In patients with IUDs, they suggested that this might consist of surface material from the IUD device (copper sulfate) plus host polypeptides. Inspissated mucous, such as that seen in endocervical glands and nabothian cysts may also provide such a nidus.

In addition to TEM and histochemistry, O'Brien *et al* used \times ray microanalysis to show that PAMRAGs are composed of copper, phosphorus, sulfur, chloride, and iron.⁵ Unlike Bhagavan,⁸ they did not identify calcium in their specimens. Based on their analyses, these authors proposed that PAMRAGs are of lipofuscin origin.⁵

Finally, Padberg and colleagues⁶ performed immunohistochemical studies that showed only a fine halo at the periphery of PAMRAGs to be positive for IgG, whereas the entire granule was negative for IgA, IgM, IgD, amyloid, and CD68. Additional TEM studies revealed cellular degradation products associated with fibrin in the centre of PAMRAGs, and peripheral phagolysosomes in neutrophils and macrophages. Given the discordance in the identification of fibrin, calcium, and immunoglobulins within PAMRAGs in the published studies, it is possible that the exact composition varies among specimens.

SUMMARY

Unlike AMGs, which contain colonies of filamentous bacteria, PAMRAGs are non-infectious, non-pathogenic entities seen in gynaecological specimens of both IUD and non-IUD users. The composition and aetiology of PAMRAGs is unresolved, despite histochemical, immunohistochemical, \times ray microanalysis, and TEM. Regardless of the inciting factors in PAMRAG formation, it remains clear that these granules should be identified and distinguished from AMGs

Table 1 Recommended panel of histochemical stains to differentiate AMGs from PAMRAGs, with expected results

Stain	AMG	PAMRAG
H&E	Irregular to spherical granules Non-refractile, granular Dense eosinophilic centre Slender peripheral filaments	Spherical granules and strips Crystalline, refractile No dense core Broad peripheral clubs
GMS	Filaments positive	Negative
B&B	Filaments positive	Strong diffuse non-specific pattern

AMG, actinomycotic granule; B&B, Brown and Brenn tissue Gram stain; GMS, Gomori methenamine silver; H&E, haematoxylin and eosin; PAMRAG, pseudoactinomycotic radiate granule.

Take home messages

- Actinomyces bacterial infection is associated with tubo-ovarian abscess and pelvic inflammatory disease, whereas pseudoactinomycotic radiate granules (PAMRAGs) do not contain bacteria and are considered non-pathogenic
- PAMRAGs may be more common in gynaecological specimens in patients with intrauterine devices than actinomycotic granules (AMGs)
- Pathologists should be familiar with the existence and diagnostic criteria of PAMRAGs to avoid an erroneous diagnosis of AMGs
- A limited histochemical panel, including haematoxylin and eosin, Brown and Brenn, and Gomori methenamine silver stains may aid in the distinction of PAMRAGs from AMGs
- Other Gram positive filamentous bacteria may be found in the gynaecological tract; therefore, culture confirmation is recommended for a definitive diagnosis of actinomyces

in gynaecological specimens. To aid in diagnosis, we recommend a limited histochemical panel, consisting of haematoxylin and eosin, Brown and Brenn, and Gomori methenamine silver stains, or their equivalent.

Authors' affiliations

B Pritt, S L Mount, K Cooper, H Blaszyk, Department of Pathology, University of Vermont College of Medicine, Burlington, VT 05405, USA

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